

CLAIMS

1. A method for determining the presence of colorectal tumors or pre-cancerous lesions in a human subject, which comprises:
 - 5 a) DNA extraction from stool samples;
 - b) PCR amplification of at least three different DNA fragments with a length of 100 base pairs or more, using deoxynucleotide triphosphates or primers labelled with detectable markers;
 - c) quantitation of the amplified fragments (amplicons);
 - 10 d) calculation of the total amount of different amplicons;
 - e) comparison of the values obtained in (d) with a reference value.
2. A method according to claim 1, wherein the detectable markers used in step (b) are fluorescent molecules.
3. A method according to claim 2, wherein said fluorescent molecules are
15 selected from HEX, 6-FAM and TAMRA.
4. A method according to claims 1-3, wherein at least 8 different DNA fragments are amplified in step (b).
5. A method according to claim 1, wherein the DNA fragments are from 100 to 500bp.
- 20 6. A method according to claim 1, wherein the DNA fragments span different regions of p53 or APC genes.
7. A method according to claim 6, wherein p53 fragments corresponding to exons 5-8 are amplified using the following pairs of primers:
 - a) ctcttcctgcagtactcccctgc; gccccagctgctcaccatcgcta;
 - 25 b) gattgctcttaggtctggcccctc; ggccactgacaaccacccttaacc;
 - c) gcgttgctctcctaggttggtctg; caagtggctcctgacctggagtc;
 - d) acctgatttccttactgcctctggc; gtctctgcttgcttacctcgcttagt;
8. A method according to claim 6, wherein APC fragments are amplified

using the following pairs of primers:

- a) aactaccatccagcaacaga; taatttggcataaggcatag;
- b) cagttgaactctggaaggca; tgacacaaagactggcttac;
- c) gatgtaatcagacgacacag; ggcaatcgaacgactctcaa;
- 5 d) cagtgatcttccagatagcc; aaatggctcatcgaggctca

9. A method according to claims 1-8, wherein the amplicon quantities are interpolated on a calibration curve obtained from known DNA amounts.

10. A method according to claim 1, wherein the amplicons are quantified with an automatic sequencer/analyser or using fluorimetric, colorimetric,
10 radioactive or spectrophotometric detection systems.

11. A method according to claim 1, wherein the reference value is determined on the basis of case series comprising healthy subjects and patients affected by colorectal tumor or lesions.

12. A kit containing oligonucleotides, labelling agents, a thermostable DNA
15 polymerase and user instructions to carry out the method of claims 1-11.